(2)

INSTITUTE REPORT NO. 210

DERMAL SENSITIZATION POTENTIAL OF GUANIDINE HYDROCHLORIDE IN GUINEA PIGS

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DIVISION OF RESEARCH SUPPORT

JANUARY 1986 **Toxicology Series 84**

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PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Dermal sensitization potential of guanidine hydrochloride in guinea pigs
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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Key words:

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Key Words: Dermal Sensitization, Toxicology, Guanidine Hydrochloride, Buehler Test, Guinea Pigs

PREFACE

TYPE REPORT: Dermal Sensitization GLP Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

WORK UNIT: 3E162720A835, Nitrocellulose-Nitroguanidine Projects
WU 180, APC TL09

GLP STUDY NO.: 84003

STUDY DIRECTOR: Don W. Korte Jr, PhD, MAJ MSC

PRINCIPAL INVESTIGATOR: Gerald F.S. Hiatt, PhD

CO-PRINCIPAL INVESTIGATOR: Earl W. Morgan, DVM, CPT VC

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Guanidine Hydrochloride

INCLUSIVE STUDY DATES: 9 May - 22 June 1984

OBJECTIVE: The objective of the study was to evaluate in guinea pigs the dermal sensitization potential of guanidine hydrochloride.

ACKNOWLEDGMENT

SP4 Paul D. Mauk, BS, SP4 Steven K. Sano, BS, and visiting scientists Max Goldman, PhD, and Joy Bauserman, MEd, assisted in the research. Yvonne C. Johnson, BS, assisted in research and statistical analysis. Richard D. Spieler, Richard Katona, Roosevelt Cunningham, and Edward M. Sands cared for the animals and managed the facility. Callie B. Crosby, BS, Lynda Araiza, and JoAnn Nishimoto provided the office management throughout the course of the study and report preparation.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84003 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

7 Jan 85

DON W. KORTE JR., PhD / DATE
MAJ, MSC
Study Director

7 Jan 85

GERALD F.S. HIATT, PhD, / DATE
DAC
Principal Investigator

7 Jan 85

EARL W. MORGAN, DVM / DATE
CPT, VC
Co-Principal Investigator

5 Jan 85

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 4 Mar 85

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

1. I hereby certify that in relation to LAIR GLP Study 84003 the following inspections were made:

14 May 84

30 May 84

19 Jun 84

21 Jun 84

2. The report and raw data for this study were audited on 1 Mar 85.

3. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 2 Jul 84 report to Management and the Study Director.

GARY L. DUTCHER
SP6, USA
Quality Assurance Unit

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Dermal Sensitization Potential of Guanidine Hydrochloride --
Hiatt et al

Nitroguanidine is being evaluated by the US Army as a replacement for the nitrocellulose component of certain propellants/munitions. The US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) has been assigned the mission of evaluating the "health effects" of nitroguanidine. As part of the mandate, USAMBRDL has tasked the Toxicology Group, LAIR, to develop a profile for nitroguanidine and intermediates/by-products of its manufacture, in accordance with the Toxic Substances Control Act regulations promulgated by the Environmental Protection Agency (EPA). One of the by-products to be tested is guanidine. The hydrochloride salt was used in this evaluation of the potential of guanidine to produce dermal sensitization.

Objective of Study

The objective of this study was to evaluate in guinea pigs the dermal sensitization potential of guanidine hydrochloride.

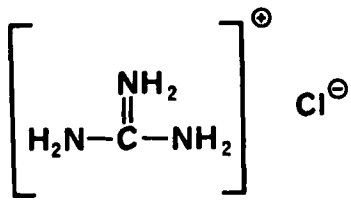
MATERIALS

Test Substance

Chemical name: Guanidine Hydrochloride

Chemical Abstract Service Registry No.: 050-01-1

Molecular structure:



Molecular formula: $CH_5N_3 \cdot HCl$

Other test substance information is presented in Appendix A.

Vehicle for Test Substance

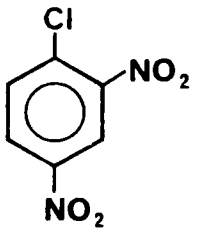
Sterile isotonic saline (Travenol Laboratories, Deerfield, IL) was used as the vehicle for guanidine hydrochloride. The expiration date for this lot (8C865A4) was December 1984.

Positive Control

Chemical name: Dinitrochlorobenzene (DNCB)

Chemical Abstract Service Registry No.: 97-00-7

Molecular structure:



Empirical formula: $C_6H_3N_2O_4Cl$

Vehicle for Positive Control

The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485) was obtained from Certified Laboratories, Inc, (Philadelphia, PA). Saline was the same as for the guanidine hydrochloride vehicle. Other positive control substance information is presented in Appendix A.

Animal Data

Forty-six male guinea pigs, Hartley strain, (Charles River Breeding Laboratories, Wilmington, MA) were studied. They were identified individually with ear tags numbered 84E0001 - 84E0046, inclusive. Two animals, the smallest and one showing mild clinical signs, were selected for quality control necropsy evaluation on receipt. Four of the animals were tested in a pilot study to determine a non-irritating dose level. Animal weights on receipt (10 May 84) ranged from 186 to 234 g. Additional animal data appear in Appendix B.

Husbandry

Guinea pigs were caged individually in stainless steel wire mesh cages in racks equipped with automatically flushing dump tanks. No bedding was used in any of the cages. The diet, fed ad libitum, consisted of Certified Purina Guinea Pig Chow Diet 5026 (Ralston Purina Company, Checkerboard Square, St Louis, MO); water was provided by continous drip from a central line. The animal room temperature was maintained in a range from 20.6°C to 24.4°C and relative humidity in a range of 33 to 74%, with occasional spikes as high as 86%. The photoperiod was 12 h of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX-82 "Buehler Dermal Sensitization Test" (1) and EPA guidelines (2).

Group Assignment/Acclimation

The guinea pigs were quarantined for 13 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Ten animals were assigned to each of four groups by a stratified randomization technique based on their body weights.

Dosage Levels

Guanidine hydrochloride was applied as a 10% solution in isotonic saline. A pilot study, using 100%, 10%, 1%, and 0.1% concentrations, indicated the 10% solution to be the highest non-irritating concentration under the conditions of this test.

Two sensitization control groups were included in the study. Dinitrochlorobenzene, a known potent sensitizing agent (3), was applied to one group, at a 0.1% concentration, as a positive control. Isotonic saline was applied to another group as a vehicle control. In addition, a negative control group received guanidine hydrochloride only on the day of challenge dosing.

Compound Preparation

Guanidine hydrochloride was readily soluble in isotonic saline. The dinitrochlorobenzene dosing solution was prepared by first adding 30 mg DNCB to 1 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

Test Procedures

The closed patch dermal sensitization test procedures utilized in this study were developed by Buehler and Griffith (4-6) to approximate the human repeated insult patch test procedures (7). Test compounds were applied for 6 h under a closed patch once a week for 3 weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site. To distinguish between reactions from primary irritation and sensitization, negative control groups were added which received only the challenge dose.

During the induction phase, the experimental, saline control, and positive control groups were dosed with 0.5 ml of the appropriate compound applied topically under a 1-in (2.5 cm) square gauze patch. This procedure was performed for three consecutive weeks (23 May, 30 May, and 6 Jun 84). The day before each dosing a 3-in (7.6 cm) square area on the left side of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp, Milwaukee, WI) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp, Stamford, CT). The patch was taped with Blenderm® hypo-allergenic surgical tape (3M Corp, St Paul, MN) to the same site each time and the animal was wrapped several times with Vetrap® (3M Corp, St Paul, MN). The patch was left in place for 6 h. When the wrap and patch were removed, the area under the patch was marked off for scoring.

Animals were challenged 2 weeks (20 Jun 84) following the third induction dose. The experimental group and the positive control group received two 0.5 ml doses, one applied to the old site on the left side and the other to a new site on the right side. Negative and vehicle control groups only received a single 0.5 ml dose which was applied to the left side. The procedures for clipping, shaving, wrapping, and exposure period remained the same.

In Buehler's procedure (4-6), skin reactions are scored 24 and 48 h after the challenge dose only. In the present study, skin reactions were scored 24 and 48 h after each induction dose as well. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema) and 3 (marked erythema). The results are expressed both in terms of incidence (the number of animals showing responses of 1 or greater at either 24 or 48 h) and severity (the sum of the test scores divided by the number of animals tested). Results from the left side are compared with right side and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-h exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cage during the exposure period. Buehler and Griffith (6) also recommended depilating the day before the challenge dose is applied. For consistency with induction procedures, this step was replaced by clipping and shaving a 3-in (7.6 cm) square area on the left side of the animals the day before dosing.

A historical listing of study events appears in Appendix C.

Deviations from Study Protocol

A 0.5 level (very slight erythema) was added to the scoring system to allow for borderline responses.

The DNCB solution was maintained at approximately 65°C before dosing. This was necessary to keep the DNCB in solution, but did not result in thermal insult to the animals' skin as the aliquot for dosing cooled quickly during pipetting and application to the patch. Significant sensitization was produced by DNCB with this method.

At the time of the first induction dose, the water supply to the animals was interrupted (0900 h 23 May to 0700 h 24 May). Close inspection of the animals immediately thereafter showed them all to be healthy and normal. No health problems or unusual behaviors were evident at this or any later time in the study.

Also at the first induction dose, one positive control animal remained patched with DNCB for approximately 22 h. This animal (84E0035) was, upon close inspection, found to be healthy and normal after patch removal. Response to DNCB in this animal was borderline (0.5 grade) at this and later times in the study.

These deviations from the protocol did not adversely affect study results.

RESULTS

Tables 1 and 2 summarize the incidence of reactions 24 and 48 h after each dose. Except for one minor response (24 h after the third induction dose guinea pig 84E0013 had a 0.5 score) there was no reaction observed in response to guanidine hydrochloride, either at 24 or 48 h.

This lack of response is reflected in Tables 3 and 4, which report the severity of skin reactions at 24 and 48 h. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. For

guanidine hydrochloride the only reponse was the 0.5 score mentioned above for animal 84E0013 following the third induction. This produced a severity index of 0.05 at 24 h.

In contrast, dinitrochlorobenzene (DNCB) produced a marked response at all time points after the first induction dose. Between 80% and 100% of the DNCB-treated animals exhibited a response 24 h following the second or third induction and challenge doses. These reactions persisted; they yielded scorable effects in 50 to 70% of the animals at 48 h after dosing.

Severity scores for these reponses to DNCB ranged from 0.7 to 1.25 at the 24 h scoring period (Table 3). The highest score, 1.25, was observed on the left (induction) side in response to the challenge dose. By 48 h the reactions had subsided somewhat, with the severity scores ranging from 0.45 to 0.6 (Table 4).

No responses whatsoever were observed in the vehicle control (saline-treated) group or in the negative control (challenge dose of guanidine hydrochloride only) group. The individual 24-h and 48-h scores for all animals appear, by group, in Appendix D.

TABLE 1

Incidences of Skin Reactions
after 24 Hours

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
Guanidine Hydrochloride	0/10	0/10	1/10	0/10	0/10
Negative Control*	---	---	---	0/10	---
Saline Vehicle	0/10	0/10	0/10	0/10	---
DNCB	0/10	9/10	9/10	10/10	10/10

* The Negative Control Group received only a challenge dose of the test compound.

TABLE 2

Incidences of Skin Reactions
after 48 Hours

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
Guanidine Hydrochloride	0/10	0/10	1/10	0/10	0/10
Negative Control*	---	---	---	0/10	---
Saline Vehicle	0/10	0/10	0/10	0/10	---
DNCB	0/10	7/10	7/10	6/10	5/10

* The Negative Control Group received only a challenge dose of the test compound.

TABLE 3

Severity of Skin Reactions
after 24 Hours

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
Guanidine Hydrochloride	0.0	0.0	0.05	0.0	0.0
Negative Control*	---	---	---	0.0	---
Saline Vehicle	0.0	0.0	0.0	0.0	---
DNCB	0.0	0.7	0.95	1.25	0.95

* The Negative Control Group received only a challenge dose of the test compound.

TABLE 4

Severity of Skin Reactions
after 48 Hours

Test Group	Induction			Challenge	
	First	Second	Third	Left	Right
Guanidine Hydrochloride	0.0	0.0	0.0	0.0	0.0
Negative Control*	---	---	---	0.0	---
Saline Vehicle	0.0	0.0	0.0	0.0	---
DNCB	0.0	0.6	0.5	0.45	0.45

* The Negative Control Group received only a challenge dose of the test compound.

DISCUSSION

Dermal Irritation and Sensitization

Most skin reactions occurring from contact with chemicals can be classified as either irritation or sensitization. Both reactions present as inflammation of the skin; the difference being the mechanism responsible for this inflammation.

Primary irritation is direct inflammation in response to injury to the skin produced by the eliciting chemical. Irritation is a locally mediated response ranging from mild reversible inflammation to severe ulceration progressing to necrosis.

Sensitization is manifested as indirect inflammation mediated by components of the immune system in response to activation by the eliciting chemical. Dermal sensitization is usually a delayed hypersensitivity or cellular immunologic reaction. During the induction phase (3 weeks in the present study) there is proliferation of a clone of T lymphocytes specifically sensitized to the eliciting antigen. Upon subsequent exposure to the antigen, these T lymphocytes release mediators, lymphokines, which initiate and amplify an inflammatory reaction at the site of contact (8).

Although both types of reactions can appear grossly similar in experimental animals, and may even be produced by the same agent, it is possible to distinguish between them. Irritation is an immediate response and can be produced upon first contact with the chemical, whereas sensitization requires at least one innocuous "conditioning" exposure before a reaction can be elicited.

Irritative responses usually require a relatively high concentration or dose of the offending chemical, while sensitization reactions may occur in response to minute quantities. Essentially all individuals in a population will express an irritative response to a reactive chemical, provided the dose is high enough, while only a fraction of the population normally becomes sensitized to the same chemical. A fully developed response can be produced by first contact with an irritant, but initial contact with a sensitizer produces no reaction (a conditioning exposure is necessary). Unless there is accumulation of damage, subsequent exposures to an irritant produce inflammation of essentially similar intensity/severity, while the reaction to a a sensitizer increases over 2 to 4 exposures after the initial contact. An irritant produces inflammation of rapid onset with short duration while a sensitization reaction is somewhat delayed and prolonged. The inflammatory response to an irritant may spread beyond the area of contact while sensitization reactions are usually circumscribed.

The features of irritation and sensitization were applied by Buehler and Griffith (4-6) to establish guidelines for differentiation between the two. In evaluating a dermal sensitization study they recommend comparing the results from a challenge dose in the experimental group with those for the negative control group:

Irritative Responses:

- occur in a large proportion of test animals.
- develop in response to the first or second exposure.
- usually fade within 24 to 48 h, unless damage is severe.
- may be stronger at challenge to a previously unexposed area of skin (contralateral flank).

Sensitization Reactions:

- occur in only a few animals, unless the compound is a potent sensitizer.
- are absent after the initial (conditioning) exposure, but appear in response to subsequent exposures.
- develop slowly, the intensity/severity of inflammation being greater at 72 to 96 h than at 24 to 48 h.
- increase in intensity/severity from one exposure to the next (at sites previously exposed or unexposed).

Dermal irritancy is evaluated by the method of Draize et al (9) in which the chemical is applied once, at high concentration, and the resulting acute inflammatory response is graded. Evaluation of sensitization potential is accomplished by repeated application, at lower non-irritating concentrations, over a few weeks. There is then a latent period, usually two weeks, to allow the immune system to elaborate and increase its specific reactivity to the chemical. A challenge dose is then given and the resulting inflammatory reaction is graded. Analysis of the incidence, severity and timing of the reaction to the challenge dose gives an estimate of the sensitizing potential of the study compound.

Guanidine Hydrochloride

In the present study, guanidine hydrochloride was evaluated for its potential to elicit a delayed-hypersensitivity reaction via dermal contact. As tested, by the Buehler and Griffith method (4-6), guanidine hydrochloride produced no response indicative of dermal sensitization. Therefore in this study, guanidine hydrochloride showed no evidence of potential to elicit an immunologic response.

Because the guinea pig exhibits a somewhat lower sensitizing responsiveness than man, the results we observed do not guarantee that guanidine hydrochloride will not sensitize humans. They do indicate that guanidine hydrochloride is unlikely to sensitize humans and the potential is low enough to permit testing in humans.

Any sensitization produced by guanidine hydrochloride would have been easily detected by this study. A hypersensitivity-type response was reliably elicted by DNCB in the present group of animals. This response to DNCB was characteristic of that observed previously within the Institute (10). Although DNCB is capable of producing primary irritation, the characteristics of responses observed in this study are indicative of a reaction due to sensitization. The concentration of DNCB used for induction and challenge is too low to produce primary irritation. Also the response to DNCB was observed only after two or more exposures and the severity generally increased with the number of previous exposures.

CONCLUSION

Guanidine hydrochloride, based on a zero percent sensitization rate in this study, exhibited no potential for inducing dermal sensitization.

RECOMMENDATION

Additional toxicological testing should be conducted on guanidine hydrochloride.

REFERENCES

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APPENDICES

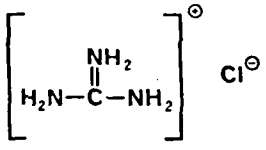
CHEMICAL DATA

Chemical Name: Guanidine Hydrochloride

Alternate Chemical Name: Aminomethanamidine hydrochloride,
Carbamamidine hydrochloride,
Carbamidine hydrochloride,
Aminoformamidine hydrochloride,
Iminourea hydrochloride

Chemical Abstracts Service Registry No.: 50-01-1

Chemical structure:



Molecular formula: CH_6ClN_3

Molecular weight: 95.5

Physical state: White powder

Melting point: 182-184°C (184-185°C*)

Analytical data/purity: Water content 0.1% by Karl Fischer analysis.*
The material is at least 98% pure and chromatographs as one spot by thin layer chromatography.†
Elemental analysis. Calculated for CH_6ClN_3
Cl, 37.1. Found: Cl, 36.6.† An IR spectrum was obtained upon receipt of the compound.
IR(KBr): 3400, 2750, 1650, 1535, 1050 (broad) cm^{-1}.
A comparison of this spectrum to the Sadtler standard spectrum confirmed the identity of the material.‡

Source: Sigma Chemical Co.
St. Louis, MO

Lot number: 103F-5623

---

*Zygmunt R., Analytical data sheet for guanidine hydrochloride, lot number 103F-5623. Sigma Chemical Co., St. Louis. 16 Feb 84.

†Sigma Chemical Company, St. Louis, MO. Becky Goodloe, PhD, personal communication, 5 March 1985.

‡Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #8676.

Stability in vehicle: A preliminary study was conducted to determine the stability of guanidine hydrochloride in the vehicle, sterile water for injection. A solution of guanidine hydrochloride (18.825 ug/ml water) was assayed after preparation and 4 hours later by using the Voges-Proskauer Method (Micklus MJ, Stein IM. The colorimetric determination of mono-and disubstituted guanidines. Anal Biochem 1973;54:545-553). This method is specific for unsubstituted and monosubstituted guanidines and yields a colored derivative which is monitored spectrophotometrically. Three samples were analyzed for each time point and the results were as follows:

Absorbance Value (1st Assay)	Absorbance Value (2nd Assay)
2.190	2.053
2.165	2.190
2.160	2.191
x = 2.172	x = 2.145

The values for the two assays were within 1.5 percent of each other which is within the error for repeated sampling using this test. This indicates that the compound is stable in aqueous solution for at least 4 hours.*

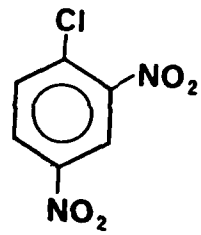
*LAIR Laboratory Notebook No. 84-05-010, pages 6-7.

Chemical Name: Dinitrochlorobenzene

Other Listed Names: 1-Chloro-2,4-dintrobenzene,
2,4 Dinitro-1-Chlorobenzene,
1,3 Dinitro-4-Chlorobenzene,
Dinitrochlorobenzol, DNCB,
Chloro-1,3-Dinitrobenzene

Chemical Abstract Service Registry No.: 97-00-7

Molecular structure:



Molecular formula: $C_6H_3N_2O_4Cl$

Molecular weight: 202.6

Physical state: Yellow crystals

Stability: Extremely stable at room temperature

Melting point: 52-54°C

Compound density: 1.7

Source: Sigma Chemical Company
PO Box 14508
St Louis, MO 63178

Lot No: 11F-0543

Purity: Approximately 95%

ANIMAL DATA

Species: Cavia porcellus

Strain: Hartley

Source: Charles River Breeding Laboratories
Wilmington, MA

Sex: Male

Date of birth: 21 April 1984

Method of randomization: Weight bias, stratified animal allocation

Animals in each group: 10 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tagging procedure, tag numbers 84E0001 to 84E0046 inclusive.

Pretest conditioning: Quarantine/acclimation 9 May - 22 May 1984

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

HISTORICAL LISTING OF EVENTS

9 May 84	Forty-six animals arrived, were examined, placed in cages, and fed.
10 May 84	Animals ear-tagged and weighed. Two animals submitted for necropsy as quality controls.
10 May-22 Jun 84	Animals checked daily.
15,22,29 May, 5,12,19 Jun 84	Animals weighed.
15 May 84	Animals randomized into groups.
15 May 84	Four pilot animals shaved. Pilot dosing solution prepared.
16 May 84	Pilot animals patch tested.
17 May 84	Pilot animals scored for 24-h skin reaction.
18 May 84	Pilot animals scored for 48-h skin reaction.
21 May 84	Pilot results evaluated, test concentration determined.
22,29 May, 5 Jun 84	Test animals, except negative control group, clipped and shaved. Dosing solutions prepared.
23,30 May, 6 Jun 84	Test animals, except negative control group, given induction dose.
23 May 84	Water supply to animals interrupted (0900 h, 23 May to 0700 h, 24 May).
24,31 May, 7 Jun 84	Test animals, except negative control group, scored for 24-h skin reaction.
25 May, 1,8 Jun 84	Test animals, except negative control group, scored for 48-h skin reaction.

Appendix C (continued)

Date	Event
19 Jun 84	Test animals clipped and shaved. Dosing solutions prepared.
20 Jun 84	Test animals given challenge dose.
21 Jun 84	Test animals scored for 24-h skin reaction.
22 Jun 84	Test animals scored for 48-h skin reaction. Forty-four animals sacrificed by injection of T-61 euthanasia solution.

APPENDIX C (concluded)

Individual Dermal Scores

APPENDIX D

TABLE 1

BUEHLER DERMAL SENSITIZATION TEST

GLP Study 84003

GROUP: ONE COMPOUND: Guanidine HCl	FIRST INDUCTION		SECOND INDUCTION		THIRD INDUCTION		CHALLENGE DOSE			
							LEFT FLANK		RIGHT FLANK	
ANIMAL NUMBER	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H
84E0004	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84E0006	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84E0008	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84E0010	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84E0013	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
84E0015	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84E0021	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84E0025	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84E0026	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84E0042	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVERAGES	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00

APPENDIX D

TABLE 2

BUEHLER DERMAL SENSITIZATION TEST

GLP Study 84003

GROUP: TWO COMPOUND: DNCB	FIRST INDUCTION		SECOND INDUCTION		THIRD INDUCTION		CHALLENGE DOSE			
							LEFT FLANK		RIGHT FLANK	
ANIMAL NUMBER	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H
84E0005	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	1.0	0.5
84E0007	0.0	0.0	0.0	0.0	1.0	0.5	1.0	0.5	1.0	0.0
84E0009	0.0	0.0	1.0	1.0	1.0	0.5	2.0	0.5	1.0	1.0
84E0016	0.0	0.0	1.0	1.0	1.0	0.0	2.0	1.0	1.0	1.0
84E0028	0.0	0.0	1.0	1.0	1.0	1.0	1.0	0.5	1.0	1.0
84E0033	0.0	0.0	0.5	0.0	1.0	1.0	1.0	0.0	1.0	0.0
84E0035	0.0	0.0	0.5	0.5	0.0	0.0	0.5	0.0	0.5	0.0
84E0036	0.0	0.0	1.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0
84E0037	0.0	0.0	0.5	1.0	1.0	0.5	1.0	0.0	0.5	0.0
84E0043	0.0	0.0	0.5	0.5	0.5	0.5	1.0	0.0	0.5	0.0
AVERAGES	0.00	0.00	0.70	0.60	0.95	0.50	0.95	0.45	1.25	0.45

TABLE 3

BUEHLER DERMAL SENSITIZATION TEST

GLP Study 84003

GROUP: THREE COMPOUND: Saline ANIMAL NUMBER	FIRST INDUCTION		SECOND INDUCTION		THIRD INDUCTION		CHALLENGE DOSE			
							LEFT FLANK		RIGHT FLANK	
	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H
84E0003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0011	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0014	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0017	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0018	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0022	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0023	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0030	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
84E0044	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA
AVERAGES	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NA	NA

TABLE 4

BUEHLER DERMAL SENSITIZATION TEST

GLP Study 84003

GROUP: FOUR COMPOUND: Negative Control	FIRST INDUCTION		SECOND INDUCTION		THIRD INDUCTION		CHALLENGE DOSE			
							LEFT FLANK		RIGHT FLANK	
ANIMAL NUMBER	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H	24 H	48 H
84E0001	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0002	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0012	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0024	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0027	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0031	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0034	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0038	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0045	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
84E0046	NA	NA	NA	NA	NA	NA	0.0	0.0	NA	NA
AVERAGES	NA	NA	NA	NA	NA	NA	0.00	0.00	NA	NA

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